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The examination of red lipsticks using microemulsion electrokinetic capillary chromatography

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ABSTRACT

Lipsticks, due to their easy transfer and widespread use, can provide important evidence in crime investigations, in particular in cases of rape, murder or burglary. Discrimination between and identification of lipstick samples is a challenge because of their similar chemical composition. The main components of lipsticks – oils and waxes – are a difficult matrix to analyse. Furthermore, lipsticks contain various additional compounds, both ionic and neutral. A promising approach to the examination of lipstick traces is the use of microemulsion electrokinetic capillary chromatography (MEEKC). In order to optimize the MEEKC method, a series of mixtures of dye standards in various microemulsions (MEs) (differed in qualitative and quantitative composition) and measurement conditions were analysed. The influence of the type and pH of the buffer, the type and amount of surfactant and oil phase, as well as the effect of the addition of a second surfactant, organic solvent and cyclodextrin, were investigated. The ME with the best separation properties contained 3% SDS, 6% butanol and 0.8% n-octane (w/w) in a borate buffer at pH 10. Short-end mode enabled the separation to be reduced to less than 4 min. Ultrasound-assisted extraction (UAE) performed for 10 min at 25 °C was found to be the most effective for real lipstick samples preparation. The developed UAE-MEEKC method was verified in terms of precision (RSD_{tm} for real samples analysed in one day was < 2.2%, and over three days was < 3.0%) and successfully used for the discrimination of six red lipsticks.

1. Introduction

Due to the growing popularity of cosmetics, there is an increasing likelihood of finding cosmetic traces at a crime scene. Smears or imprints of lipstick can provide significant forensic evidence in cases of rape, burglary or murder because of the mentioned commonness of use and ease of transfer. They can be used as indirect evidence of 1) a link between the suspect or victim and the site of the incident, or 2) contact between the suspect and the victim. Examining this type of alternative material is particularly important due to the fact that lipstick smears/imprints are microtraces. A criminal can easily overlook them or remove them in an incomplete way, providing material that can constitute fundamental evidence in an investigation. It is therefore important to develop research methods that would allow a credible analysis of this type of evidence.

However, identifying traces of red lipsticks and their differentiation can be a serious challenge. Most products of this type have a very similar chemical composition. Their main ingredients – oils and waxes (content in lipstick up to 80%) – constitute a matrix that is difficult to analyse. Additionally, lipsticks include a wide range of compounds exhibiting a different chemical nature, both neutral and ionic, differing in water solubility and/or polarity. The rich composition of lipsticks makes it difficult to find a technique that would allow the simultaneous analysis of all the substances contained in them.

There are a lot of modern analytical methods (non-destructive [1–9] and destructive ones [1,10–19]) which have been applied in the forensic examination of lipsticks. To the best of the authors' knowledge, there are three articles reported the results of the utilization of capillary electrophoresis (capillary zone electrophoresis (CZE) [20] and micellar electrokinetic capillary chromatography (MEKC) [21,22]) in forensic analysis of lipstick.

In authors opinion, the use of microemulsion electrokinetic capillary chromatography (MEEKC) is also a promising prospect for analysing lipstick traces. It is an another mode of the capillary electrophoresis technique in which a microemulsion (ME) solution is used as the background electrolyte (BGE). It is a thermodynamically stable dispersion system, consisting of four main components: water phase, oil phase, surfactant, and co-surfactant, which must be mixed in appropriate proportions. The use of ME as the BGE means that not only electrophoretic and chromatographic interactions appear in the

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measuring system, but also electrostatic ones – attraction or repulsion – between the ME and analytes contained in the sample. Variation of many ME components can influence the MEEKC separation process and provide several promising options for development of the method [23–29] in particular: 1) the type of surfactant may affect the oil droplet charge and size, the level and direction of the EOF, and ion-pair interactions; 2) the type of buffer may affect the level of current generated (e.g. a low-ionic strength zwitterionic buffer such as Tris generates minimum current, which allows higher voltages to be applied and high-speed separation to be achieved); 3) in order to speed up the separation (by applying higher voltages), the oil commonly used to prepare ME (n-octane) can be replaced by ethyl acetate, which has a lower surface tension and requires lower SDS content to form a stable ME; 4) the addition of a second surfactant to the ME can also improve the method's selectivity and resolution, reduce the analysis time, and generally increase the efficiency of the electrophoretic separation process; 5) in order to obtain a positive effect on MEEKC separation, non-ionic surfactants can be added to the ME, even in higher concentrations than ionic surfactants, without increasing the overall operating current; and 6) the addition of an organic solvent can significantly improve the efficiency or selectivity of the electrophoretic separation process.

Due to its unique properties, MEEKC – since it was established by Watari in 1991 [30] – has constantly aroused the interest of scientists from around the world. The number of publications about the MEEKC technique is growing due to the constant development of new, increasingly perfect methods and technological advances. In the literature, one can find a multitude of articles summarizing a wide range of applicability of this technique in various fields of life and science [31–43]– from basic tasks, such as the separation of simple mixtures of different kinds of analytes, through determination of substance partition coefficients, to complicated analyses of chiral mixtures. The above mentioned reviews clearly show that MEEKC can be extensively used concerning various compounds and is especially useful for complex mixtures containing analytes with varying charge and hydrophobicity. From the point of view of the purpose of this study – the separation of samples of waxy/oily matrix – the successful application of MEEKC to various nonpolar substances, such as β-diketones, poly-aromatic hydrocarbons, steroids, polymer additives, fatty acids, and lipid-soluble vitamins [35,44–46] is especially important. A very interesting article is one that reported the development of MEEKC methods (using anionic and cationic MEs) for the separation of paracetamol and its impurities [47]. The presented method gave excellent validation results for paracetamol content in suppositories (containing hard fat and polyoxyl 40 stearate as excipients). A novel MEEKC method using CTAB MEs enabled separation of all of the impurities. This method also provided significant benefits in terms of reduced sample pre-treatment requirements. CTAB MEs had greater solubilising power than their SDS equivalents and were more stable due to their longer alkyl chains.

Bearing in mind that apart from waxes and oils the main components of lipsticks are dyes, the capabilities of MEEKC with regard to this type of analytes were also considered. There are two articles in which the results of determination of food colorants by MEEKC have been presented [48,49]. Some of the most used food colorants (Tartrazine (E102), Sunset Yellow (E110), Red Allure (E129), Carmine (E120), and Blue Brilliant (E133)) are, indeed, organic dyes that are also found in lipsticks. Thus, these articles confirm that the MEEKC method is a useful tool in such an application and a rapid, reliable and versatile MEEKC method for colorant analysis can be developed.

MEEKC is certainly a viable alternative: because of its enhanced solubilisation capacity and broadened elution range, it allows for the baseline resolution of certain hydrophobic analytes in a short time [24,35]. In the case of a very small amount of material available for investigation (a common situation in forensic examinations), this enhanced solubilisation capacity – allowing omission of the multi-stage process of sample preparation that carries a risk of contamination – is an important advantage.

Thus, the main goal of this study was to develop and optimize an MEEKC method dedicated to the analysis of lipsticks that has to be characterized by: 1) a sample preparation procedure that has been reduced to a minimum, and requires only a small amount of sample, but is still repeatable, and 2) a rapid, precise and robust separation procedure allowing reliable separation of a wide range of analytes with high efficiency and resolution power. The optimization process was performed comprehensively, including every step of the analytical procedure: sample preparation, conditioning/rinsing of capillary, injection, separation, and detection. At each of the above-mentioned stages, there is a whole group of phenomena that can have a profound effect on the results obtained. Understanding and exploring the basic phenomena and relationships between many physical variables influencing the MEEKC analysis would undeniably help improve the selectivity, precision, and sensitivity of the proposed method [50].

2. Experimental part

2.1. Instrumentation

The following apparatuses were used to carry out all the experiments: P/ACE™ MDQ capillary electrophoresis system (Beckman Coulter, USA) equipped with a photo diode array detector (190–600 nm); XUBA3 ultrasonic bath (Grant Instruments, England); Sonic-3 ultrasonic bath (Polsonic, Poland); XA220/X analytical balance (Radwag, Poland); Mili–Q water purification system (Merck – Millipore, Germany); Microfuge 16 centrifuge (Beckman Coulter, Germany); automatic pipettes (Hirschmann, Germany; Sartorius, Germany). CE instrument control, data acquisition and evaluation were accomplished with 32Karat version 8.0 (Beckman Coulter, USA).

2.2. Materials and samples

The chemicals used throughout the experiments: acetonitrile (ACN), Brij-35, hexadecyltrimethylammonium bromide (CTAB), sodium cholate, β cyclodextrin, sodium dihydrogen phosphate, sodium dodecyl sulphate (SDS), sodium docusate, acetic acid, methanol, sodium acetate, propan-2-ol (isopropanol), sodium tetraborate (borax), tris (hydroxymethyl) aminomethane (Tris), Triton X-100, Tween 20, and sodium hydrogen phosphate were supplied by Sigma-Aldrich (Poland). Butyl alcohol (butanol), ethyl acetate, and hydrochloric acid 37% were purchased from Merck (Poland). Sodium hydroxide 30% was bought in POCH (Poland) and n-octane in VWR (Poland). All reagents and solvents were LC-MS grade or p.a. purity. Ultrapure water (18.2 MΩ cm, $<$ 3 ppb TOC) was generated in the laboratory with the Milli-Q system by Merck-Millipore (Germany). The composition of pH buffers used in this study was calculated using PHoEBuS 1.3 software by Analis (Namur, Belgium). Their particular compositions are presented in Table 1 in the Electronic Supplementary Materials (ESM).

Optimization of the composition of the ME was carried out using eight dye standards: Acid Red 33 (CI 17,200, dye content (dc) $\geq$ 97%), Carmine (CI 75,470, dc $\geq$ 85%), Eosin Y disodium salt (CI 45,380, dc $\geq$ 85%), Orange II (CI 15,510, dc $\geq$ 85%), Rhodamine B (CI 45,170, dc $\geq$ 97.0%), Sunset Yellow FCF (CI 15,985, dc $\geq$ 90%), and Tartrazine (CI 19,140, dc $\geq$ 99.0%) purchased in Sigma-Aldrich (Germany), and Red 6 sodium salt – donated by Inglot Sp. z o.o. (Poland). The choice of standard dyes was dictated by information found in the literature [51], in the descriptions of lipstick compositions found on packaging or on various (scientific or popular scientific) websites concerning cosmetics. The structural formulas of all the dyes used are presented in Figure I in the ESM. A mixture of eight dyes was prepared using 2 μL of standard stock solution (1 mg mL^{-1} in MeOH). 16 μL of dye mixture was transferred to a 250 μL PCR tube, mixed, and 100 μL of ME was added. Before measurements, samples of dye standard mixture were degassed in an ultrasonic bath (10 min, room temperature).

There were six red lipsticks examined in this study: E 02 Longlasting

Table 1
Conditions of separation process in standard and short-end mode.

Parameter	Standard mode	Short-end mode
Capillary		
Total length	31.0 cm	31.0 cm
Length to the detector	20.8 cm	10.2 cm
Inner diameter	50 μm	50 μm
Outer diameter	375 μm	375 μm
Temperature		
Capillary	25 °C	50 °C
Sample module	10 °C	12 °C
Separation		
Time	15 min	5 min
Voltage	20 kV	−20 kV
Sample injection		
Mode	Hydrodynamic	Hydrodynamic
Time	6 s	2 s
Pressure	0.7 psi	0.3 psi

Lipstick (L08); L'Oreal Paris 330, color Riche (L10); Maybelline New York 955, color Sensational (L11); Catrice 440, Ultimate color (L17); Catrice 140, Ultimate Stay (L18), and Eveline Cosmetics 710, color Edition (L23). They were randomly selected from 43 items collected in our laboratory (Laboratory for Forensic Chemistry) [22]. All lipstick samples were prepared according to the following procedure. A 200 μL pipette tip was immersed to a depth of 1 mm in a lipstick. The outer walls of the tip were wiped with dust-free paper. 50 μL of ME was added to the tip and the lipstick was pushed, using a pipette, into an extraction vial. Then, the sample was subjected to ultrasound-assisted extraction (UAE) and later centrifugation. After that, about 30 μL of the extract was taken, transferred to a 250 μL PCR tube. Optimization of the UAE and centrifugation conditions are described later in *Results and discussion.*

2.3. Methods

In order to optimize the ME composition, the electrophoretic separation process was initially carried out under the instrumental conditions presented in Table 1 (standard mode).

3. Results and discussion

3.1. Optimization of the qualitative and quantitative composition of the microemulsion

The formation process and stability of the ME depend on how it is prepared as well as on the types and proportions of its components. A properly prepared ME takes the form of a clear liquid, capable of retaining its properties for many weeks (or months).

In the initial stage of this study, the ME preparation (order of added components, effect of ultrasonication and filtration) and composition were optimized. The best way to prepare ME was found to be as follows: the organic liquid components, co-surfactant and the organic phase were consecutively weighed in an empty, dry, clean beaker (150 mL). Next, a surfactant was added, trying to place it directly into the organic phase. After circular mixing, the aqueous phase was added (buffer). The beaker, which was covered with Parafilm sealing foil (Brand, Germany), was put into an ultrasonic bath for 30 min at room temperature. Then, the ME was filtrated using a nylon filter (0.45 μm). Each ME was prepared at least 24 h before the planned measurement and kept in dark glass bottles. Details of the composition of all MEs considered in this study are listed in Table 2.

When optimizing the separation efficiency, the choice of the most promising ME was based on visual assessment of the obtained electropherograms, comparing migration times, number, shape and resolution of the peaks. First, the type of buffer and its pH as well as the type of surfactant and its quantity were examined. An anionic surfactant, sodium dodecyl sulphate (SDS), and a cationic surfactant enabling ion-pair interactions, i.e. hexadecyltrimethylammonium bromide (CTAB), were taken into consideration. A series of MEs containing 3% SDS in buffers at pH ranging from 2.5 to 10 (ME1 – ME10) and containing 3% CTAB in buffers at pH ranging from 2.5 to 8 (ME11–ME16) were prepared (see Table 2). It is worth noting that CTAB creates a positive layer on silanol groups of silica (capillary walls), so the EOF is reversed, and reverse electrode polarization was applied. In the case of MEs with anionic SDS, it was observed (Fig. 1A) that with lower pH, the current stability and repeatability of the results decreased, the electropherograms were characterized by an increasingly disturbed electrophoretic profile, and the resolution was significantly poorer. The best electropherogram (at 220 nm) was obtained in ME2 (pH 10) – 7 intense, well-shaped peaks coming from dyes were visible (two overlapping signals at about 3.9 min could be distinguished using a PDA image – different wavelengths). The baseline did not show significant noise and the analysis time was the shortest. When using CTAB MSs, unfortunately, no satisfactory results were observed (data not presented). Regardless of pH, the results showed a significant baseline increase and disturbance in the first minutes of measurement, and additionally, very poor resolution.

In the next experiment, MEs were used with Tris buffer (pH 8.1) instead of borate buffer (ME17 – ME22), with ethyl acetate instead of n-octane (ME23), and with both Tris and ethyl acetate (ME24, ME25), together allowing a significant reduction of the surfactant content (to 0.6% w/w). The MEs were prepared (details in Table 2) and the mixture of 8 dyes was separated using them. In these cases, higher voltages could be applied to the system. The most satisfactory result was achieved using ME23 with 0.6% SDS: the peaks were relatively high and narrow, and the analysis time was only 0.5 min longer than for ME2 (see Fig. 1B). However, an in-depth comparison revealed that there is no improvement in resolution - only seven peaks from dyes could be found in both electropherograms (ME2 and ME23).

In order to investigate the influence of different surfactants on the migration and separation selectivity, SDS was mixed (1:1 w/w%) with another anionic surfactant, sodium docusate (SD) (ME26), and with neutral surfactants such as Brij-35, Tween 20, and Triton X-100 (ME27, ME28, and ME29, respectively). The results are shown in Fig. 2A. The addition of SD impaired the separation efficiency: the peaks lost their intensity and the baseline became less regular. Only six of the visible peaks were from dyes, so resolution deteriorated. The addition of neutral Brij-35 to ME also deteriorated the separation efficiency: resolution decreased drastically; furthermore, the number and intensity of peaks decreased while their width increased. The Triton X-100 (a compound that absorbs UV–vis radiation) signal recorded by the detector was so strong that it interfered with the signals coming from the dyes. The addition of Triton X-100 prevented reliable interpretation of the electropherogram obtained. Better results were observed when neutral Tween 20 (ME28) was added: although the resolution also deteriorated, the analysis time was significantly reduced (almost halved). However, in the electropherogram (at 220 nm), only 5 intense, well-developed peaks are visible.

MEs containing an additional organic component were prepared in order to check if the separation capacity of ME2 would increase. However, in some cases, problems with the formation of transparent solutions were encountered. MEs with a 15% w/w addition of ACN (ME30) and MeOH (ME31) were not formed, while an ME with the addition of isopropanol in the same w/w ratio (ME32) was successfully prepared. A decrease in organic additive content to 8% also enabled preparation of ME with ACN (ME34) and MeOH (ME35). As can be seen in Fig. 2B, promising results were obtained only with the addition of 8% of isopropanol (ME36). For the other reagents, a significant deterioration in resolution, evidenced by a small number of peaks was observed. The addition of isopropanol, indeed, improved the separation efficiency of ME28 (with Tween 20). However, although in the case of ME36 the

Table 2
Composition of microemulsions (MEs) analysed in this study.

Name	Surfactant		Oil	Cosurfactant	Buffer			Organic additive	Has it formed?
	1	2			pH	IS*	type		
ME1	3% SDS	–	0.8% n-octane	6% butanol	9	10	borate	–	YES
ME2	3% SDS	–	0.8% n-octane	6% butanol	10	10	borate	–	YES
ME3	3% SDS	–	0.8% n-octane	6% butanol	8	10	phosphate	–	YES
ME4	3% SDS	–	0.8% n-octane	6% butanol	7	10	phosphate	–	YES
ME5	3% SDS	–	0.8% n-octane	6% butanol	6	10	phosphate	–	YES
ME6	3% SDS	–	0.8% n-octane	6% butanol	5	10	phosphate	–	YES
ME7	3% SDS	–	0.8% n-octane	6% butanol	4	10	acetate	–	YES
ME8	3% SDS	–	0.8% n-octane	6% butanol	2.5	10	phosphate	–	YES
ME9	3% SDS	–	0.8% n-octane	6% butanol	2.5	10	phosphate	–	YES
ME10	3% SDS	–	0.8% n-octane	6% butanol	2.5	54	phosphate	–	YES
ME11	3% CTAB	–	0.8% n-octane	6% butanol	8	10	phosphate	–	YES
ME12	3% CTAB	–	0.8% n-octane	6% butanol	5	10	acetate	–	YES
ME13	3% CTAB	–	0.8% n-octane	6% butanol	4	10	acetate	–	YES
ME14	3% CTAB	–	0.8% n-octane	6% butanol	2.5	54	phosphate	-	YES
ME15	3% CTAB	-	0.8% n-octane	6% butanol	2.5	54	phosphate	–	YES
ME16	3% CTAB	–	0.8% n-octane	6% butanol	2.5	10	phosphate	–	YES
ME17	0.6% SDS	–	0.8% n-octane	6% butanol	8.1	–	Tris	–	NO
ME18	0.6% SDS	–	0.5% n-octane	1.2% butanol	8.1	–	Tris	–	NO
ME19	3% SDS	–	0.8% n-octane	6% butanol	8.1	–	Tris	–	YES
ME20	0.75% CTAB	–	0.8% n-octane	6% butanol	8.1	–	Tris	–	NO
ME21	0.75% CTAB	–	0.5% n-octane	6% butanol	8.1	–	Tris	–	NO
ME22	3.8% CTAB	–	0.8% n-octane	6% butanol	8.1	–	Tris	–	YES
ME23	0.6% SDS	–	0.5% ethyl acetate	1.2% butanol	10	10	borate	–	YES
ME24	0.6% SDS	–	0.5% ethyl acetate	1.2% butanol	8.1	–	Tris	–	YES
ME25	1.2% CTAB	–	0.5% ethyl acetate	1.2% butanol	8.1	–	Tris	–	YES
ME26	1.5% SDS	1.5% sodium docusate	0.8% n-octane	6% butanol	9	10	borate	–	YES
ME27	1.5% SDS	1.5% Brij-35	0.8% n-octane	6% butanol	9	10	borate	–	YES
ME28	1.5% SDS	1.5% Tween 20	0.8% n-octane	6% butanol	9	10	borate	–	YES
ME29	1.5% SDS	1.5% Triton X-100	0.8% n-octane	6% butanol	9	10	borate	–	YES
ME30	1.5% SDS	1.5% Tween 20	0.8% n-octane	6% butanol	10	10	borate	15% acetonitrile	NO
ME31	1.5% SDS	1.5% Tween 20	0.8% n-octane	6% butanol	10	10	borate	15% methanol	NO
ME32	1.5% SDS	1.5% Tween 20	0.8% n-octane	6% butanol	10	10	borate	15% isopropanol	YES
ME33	1.5% SDS	1.5% Tween 20	0.8% n-octane	6% butanol	10	25	borate	–	YES
ME34	1.5% SDS	1.5% Tween 20	0.8% n-octane	6% butanol	10	25	borate	8% acetonitrile	YES
ME35	1.5% SDS	1.5% Tween 20	0.8% n-octane	6% butanol	10	25	borate	8% methanol	YES
ME36	1.5% SDS	1.5% Tween 20	0.8% n-octane	6% butanol	10	25	borate	8% isopropanol	YES
ME37	3% SDS	–	0.8% n-octane	6% butanol	10	10	borate	3% isopropanol	YES
ME38	3% SDS	–	0.8% n-octane	6% butanol	10	10	borate	1% isopropanol	YES
ME39	3% SDS	–	0.8% n-octane	6% butanol	10	10	borate	0.7% β cyclodecstrin	YES
ME40	3% sodium cholate	–	0.8% n-octane	6% butanol	10	10	borate	–	YES

* ionic strength.

analysis time was about 0.8 min shorter compared to ME2, the same number – seven – of peaks originating from dyes could be observed (see Fig. 2B). So, an measureable advantage of using ME2 was the higher intensity of the peaks obtained.

To confirm the conclusions drawn on the basis of visual observation of the electropherograms, the *F* function was proposed by authors and calculated for the four most promising MEs (ME2, ME23, ME28 and ME36). This criterion was defined by the formula $F = nR/t$, where: n is the number of all peaks (not only corresponding to dyes) present in the electropherogram with an intensity at least three times higher than the noise level, R is the resolution, and t is the migration time of the last peak. The higher the value of the *F* function, the better the separation properties of the ME. When choosing the best ME, both the electrophoretic profile and the calculated *F* value were taken into account. So far, the best properties were shown by ME2, which was also confirmed by *F* equal to 2.4 (in contrast to $F_{ME23} = 1.5$; $F_{ME28} = 2.0$; $F_{ME36} = 1.9$).

3.2. Optimization of electrophoretic separation conditions in short-end mode

The analysis time for ME2 with the best separation properties was less than six minutes (time of the last peak). This is quite satisfactory, but it was decided to make it even shorter using MEEKC in short-end mode. In order to achieve high precision, the following factors were checked: the capillary rinsing between measurements, the capillary conditioning prior to analyses, the sample injection, and the capillary and sample module temperature. Conditioning of the capillary tube, both when a new capillary is used and before the day of analysis begins, allows the appropriate chemical character of the capillary inner wall to be obtained, and thus ensures high repeatability of results. Using the mixture of eight dyes (see Section 2.2), two different methods of conditioning the capillary before and three different methods of rinsing it between measurements were examined (Table 2 in ESM), and the relative standard deviations of migration times (RSD_{tm}) were calculated (data not shown). On this basis, the better methods in terms of cleaning the capillary walls were selected for further research (for details of daily conditioning and rinsing, see Table 3). Information about the first conditioning of a new capillary and rinsing after the sequence of runs are also presented in this table.

The next step of optimization focused on sample injection. A volume of injected sample that was as large as possible but did not disturb the separation process and gave, simultaneously, peaks of the maximum intensity, was sought. 15 variations of applied pressure (0.3–0.7 psi, step 0.2 psi) and time (2–6 s, step 1 s) of hydrodynamic injection were analysed (Table 3 in ESM). The analysis was carried out with the use of the same 8-dye mixture but prepared in a double amount to avoid the effect of evaporation on the results obtained. A clear tendency was

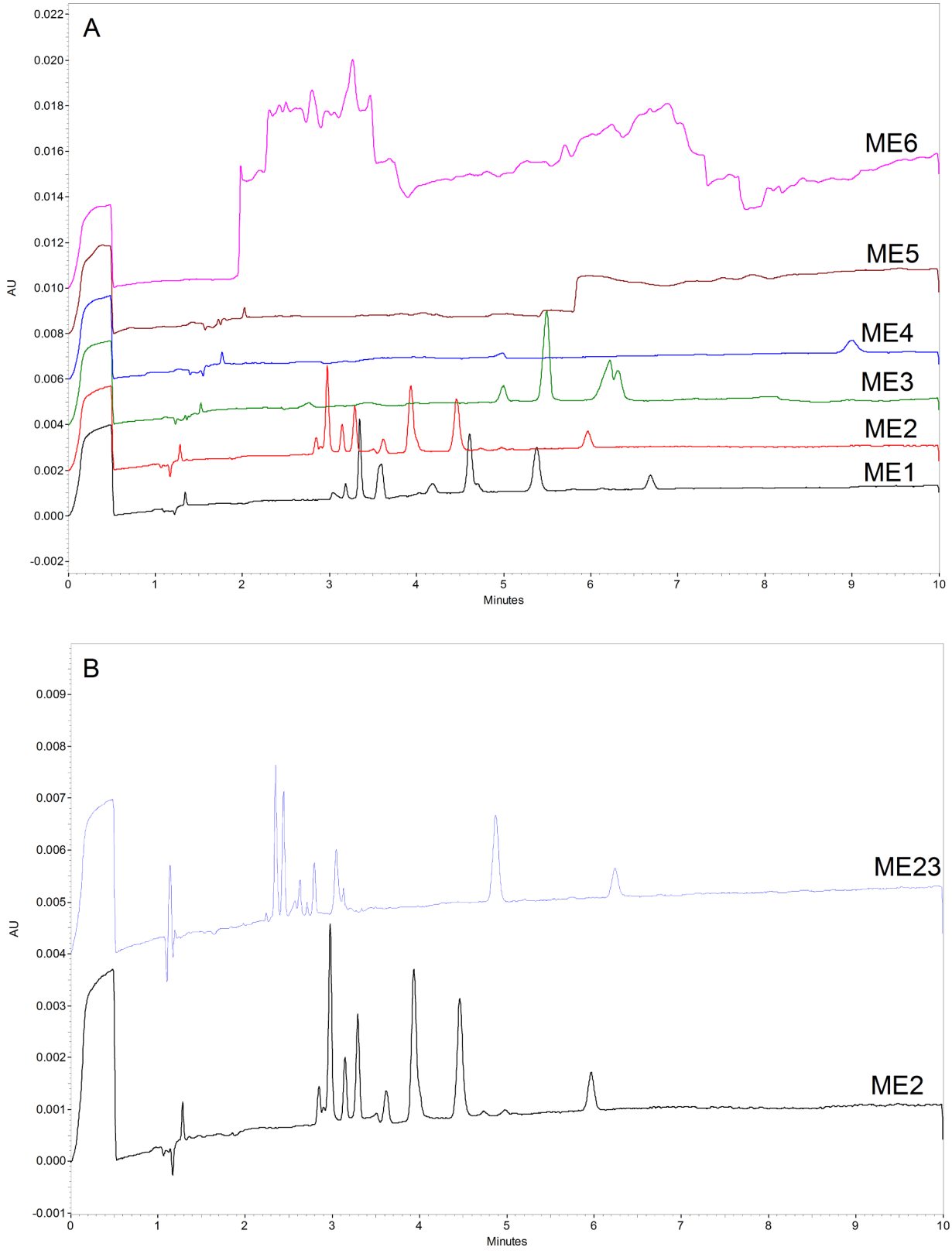

Fig. 1. A) Electropherograms at 220 nm of 8-dye mixture obtained in MEs containing 3% SDS, 6% butanol, 0.8% n-octane in buffers at pH: 10 (ME2), 9 (ME1), 8 (ME3), 7 (ME4), 6 (ME5), and 5 (ME6); B) Electropherograms at 220 nm obtained for 8-dye mixture in MEs containing: 1) 3% SDS, 6% butanol, 0.8% n-octane, buffer pH 10 (ME2), and 2) 0.6% SDS, 1.2% butanol, 0.5% ethyl acetate, buffer pH 10 (ME23).

noticed – the higher the volume of injected sample, the worse the electrophoretic profiles (the wider and the less intense the peaks). The optimal parameters were considered to be 2 s at a pressure of 0.3 psi.

To investigate the effect of temperature on the separation process, the mixture of eight dyes was analysed at 30, 40 and 50 °C using ME2 and the temperature of 50 °C was chosen as the most favourable. The last peak originating from dye appeared after 3.2 min, so the analysis time had been reduced (from about 6 min) without loss of resolution. At

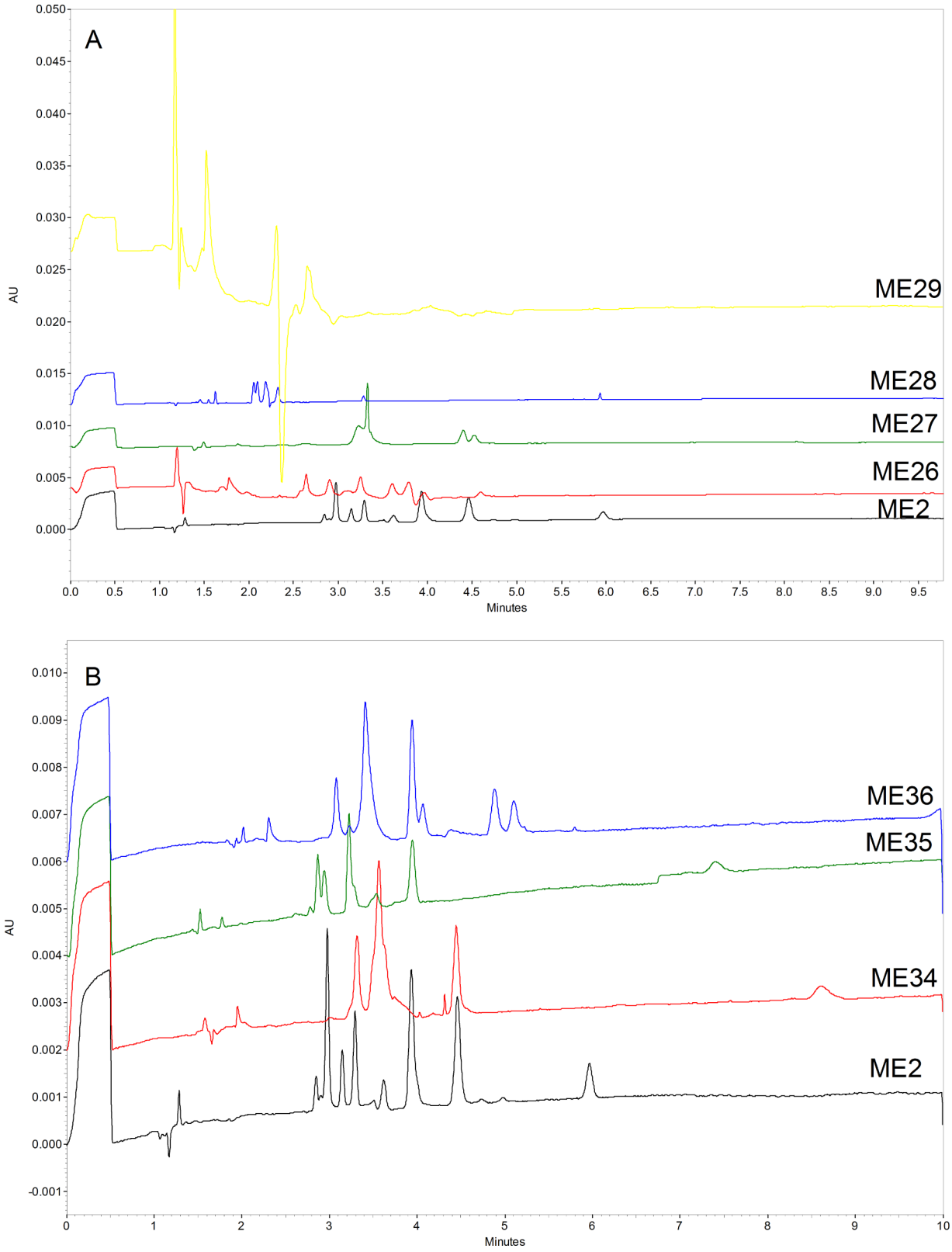

Fig. 2. A) Electropherograms at 220 nm of 8-dye mixture in MEs containing 1.5% SDS, 6% butanol, 0.8% n-octane in borate buffer, and with addition of 1.5% (w/w): sodium docusate (ME26), Brij-25 (ME27), Tween 20 (ME28) and Triton X-100 (ME29). B) Electropherograms at 220 nm of 8-dye mixture obtained in MEs of 1.5% SDS, 1.5% Tween 20, 6% butanol, 0.8% octane with: 8% methanol (ME34), 8% acetonitrile (ME35) and 8% isopropanol (ME36). The electropherogram obtained in ME2 is presented for comparison.

this temperature, a certain oscillation of the current value (about ± 0.15 μA) occurred. However, these oscillations did not disrupt the repeatability of the measurements, as RSD_{tm} did not exceed 0.4% for all peaks except the last one (1.3%).

The temperature of the sample module was also tested and optimized. To avoid evaporation of volatile components of a sample (which

Table 3
The optimal capillary conditioning and rinsing conditions.

No.	Solution	Time (min)			
		First conditioning	Daily conditioning	Rinsing	End rinsing
I	Methanol	10	5	5	5
II	Ultra-pure water	2	–	–	–
III	1 M HCl	5	5	2	2
IV	Ultra-pure water	2	1	1	1
V	0.1 M NaOH	10	10	2	2
VI	Ultra-pure water	2	1	–	1
VII	Microemulsion (BGE)	10	10	1.5	–
VIII	Air	–	–	–	1

was diluted in ME) during the long sequence of analysis, as well as other changes in a sample (e.g. solidification), four temperatures of 10, 12, 15 and 25 °C were tested. According to the obtained results, 12 °C was the optimal temperature. RSD_{tm} for all peaks of the 8-dye mixture (5 repetitions) was less than 2.5%, while, for instance, at 15 °C it reached 5.5%.

Using short-end mode in the above-selected optimal conditions (see also Table 1), the 8-dye mixture was separated in ME2 (Fig. 3). The electropherogram profile (at 220 nm) was retained but the analysis time was reduced (the migration time of the last peak was equal to 3.2 min). The resolution decreased very slightly. Two peaks at about 2.1 min overlapped, however, in photo diode array (PDA) image, 8 signals originating from dyes were visible (530 nm was suggested as alternative wavelength to detect the presence of one of the coeluting dyes – see Fig. 3C). The UV-vis spectra of the unknown dyes were compared to the spectra from database and according to the best match, the electrophoretic order of the 8 applied dyes was established (Table 4 in ESM). It is worth noting here that the intensity of peaks was lower than in the standard mode of CE. Therefore, an attempt was made to improve the intensity and separation efficiency by adding an organic modifier (isopropanol, ME37, ME38) and β cyclodextrin (most often used in MEEKC methods because of its wide usability and relatively low price [52], ME39), and by changing the surfactant to a bile salt surfactant (sodium cholate, ME40). Unfortunately, neither the resolution nor the intensity of the obtained peaks was improved (data not shown).

3.3. Optimization of lipstick extraction

Lipstick samples were prepared according to the procedure presented in Section 2.2. Optimization of UAE conditions was carried out, modifying the temperature and time of the process. Seven experimental points were examined (Table V in ESM). One of the main goals of this study was to develop the simplest extraction method – not requiring many steps and thus minimizing the likelihood of contamination. Therefore, two randomly selected lipsticks, L10 and L15, were extracted using ME2 (the optimal BGE) as the extracting agent. This choice was also caused by the fact that the sample extractant/diluent has a huge impact on the correct course and precision of MEEKC separation [5]. The results revealed that the extraction process performed for 10 min at 25 °C had a similar efficiency to a longer process carried out at a higher (and consequently unstable) temperature. Thus, this condition was chosen as the best one.

It was observed that sample centrifugation (needed for degassing and cleaning of solid micro particles) can have an impact on the ME properties, so optimization of the centrifugation conditions of the extracted samples was also carried out. Sample L10 (extracted in the above-mentioned optimal conditions) was centrifuged for five minutes at 3000, 6000, 9000, 12,000 and 14,800 rpm. According to the obtained MEEKC results (uninterrupted separation, stable baseline), the optimal centrifugation of the sample is 12,000 rpm for 5 min.

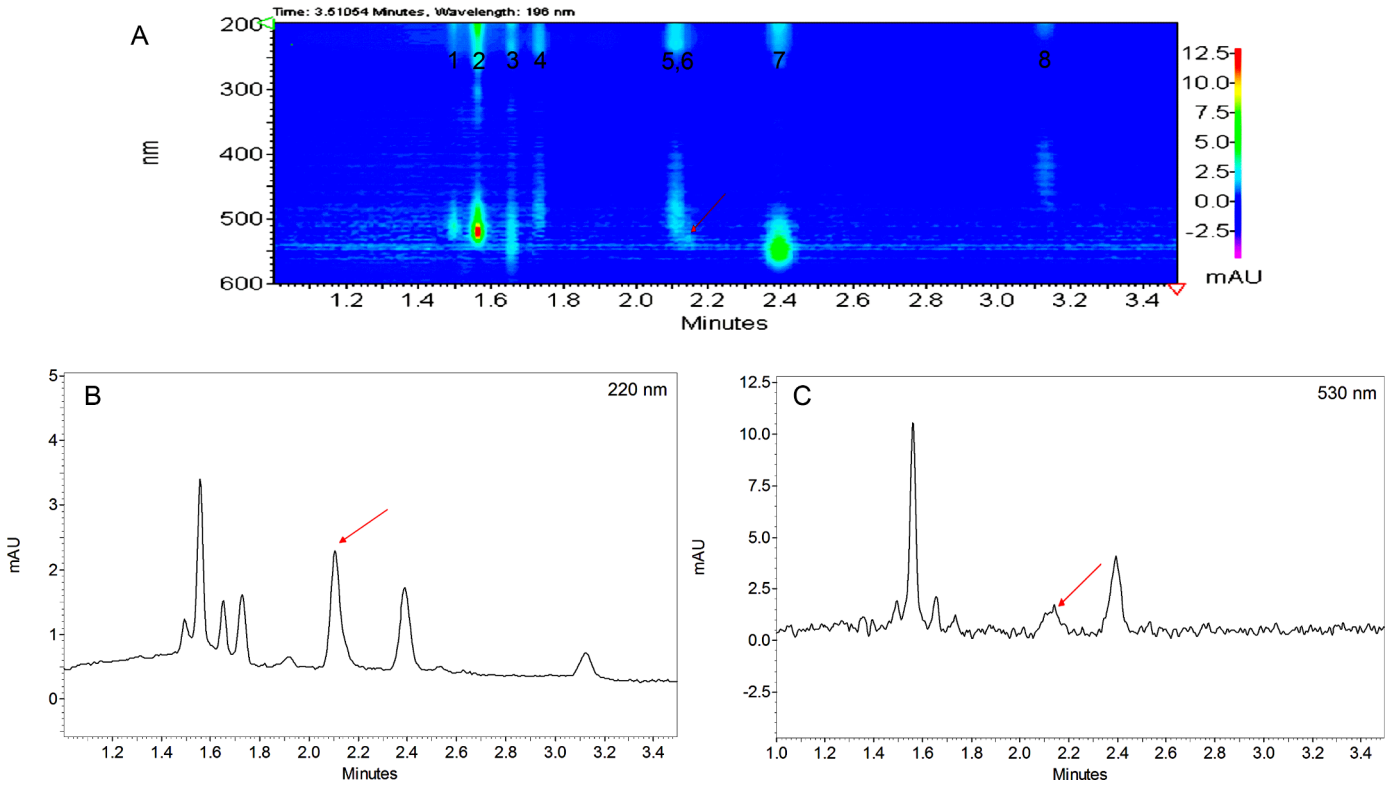

Fig. 3. A) PDA image, B) electropherogram at 220 nm, C) electropherogram at 530 nm; obtained in ME2 in short-end mode. Overlapping peaks are marked with arrows.

3.4. Evaluation of the UAE-MEEKC method

The aim of the research was to develop an analytical method applicable to the analysis of red lipsticks for forensic purposes. Such examinations are focused, as a rule, on qualitative analysis, in which evidence is compared with reference material or with data from a database for the purpose of discrimination or group identification of the trace being investigated. When verifying the correctness of the method, the following parameters were determined: the injection repeatability, the sampling repeatability, the intermediate precision of sampling, and the intermediate precision of the method with respect to the capillary.

Sample L10, prepared in accordance with the methodology presented in Sections 2.2 and 3.3, was analysed. To determine the repeatability of the injection, five measurements of one sample were taken, while to determine the repeatability of the sampling (preparation + extraction + separation), one analysis was carried out for each of five samples. The intermediate precision of sampling was determined by analysing each of five samples once a day over three days, while the intermediate precision of the method with respect to the capillary was determined by measuring each of five samples using two capillaries (10 measurements total). The average migration times and the RSD_{tm} were calculated for the five most intense peaks. The results are shown in Table 6 in ESM.

Based on these results (Table 6 in ESM), it can be concluded that the developed method is characterized by satisfactory precision. The RSD_{tm} had the lowest values for the repeatability of injection, not exceeding 1.7%. For sampling repeatability, the RSD_{tm} was less than 2.2%, while for intermediate precision of sampling, the RSD_{tm} did not exceed 3.0%, which is a satisfactory result for real samples. Nevertheless, it can be stated that sampling (both the repeatability of the portion taken and the selected location on the lipstick stick) has the greatest impact on the precision of the extraction process. The worst results were obtained for intermediate precision with respect to capillary: the highest RSD_{tm} value was 4.3%. Therefore, to obtain the most precise results, analyses should be performed on the same capillary and preferably during one day.

3.5. Analysis of commercially available red lipsticks

The optimized MEEKC method was used to analyse six red lipsticks: L08, L10, L11, L17, L18 and L23 (details in Section 2.2). Samples were taken and prepared in accordance with the procedure described in Sections 2.2 and 3.3 and injected into the CE instrument under the conditions specified in Table 1 (short-end mode). The electropherograms at 220 nm for the analysed lipsticks are presented in Fig. 4A.

Four lipsticks, L08, L11, L18 and L23, could be definitely distinguished based on the absence or occurrence of peaks characteristic for the given lipstick. For instance, only the electropherogram of lipstick L11 did not have a peak at about 2.05 min (coming from yellow dye), whereas an intense peak at 1.2 min was only visible for the L18 lipstick. Two peaks were identified with the use of the dye spectra database (i.e. a peak occurring in L08, L10, L11, and L17 at 1.55 min originating from Eosin Y, and a peak at about 1.7 min visible only in L10, L11 and L17, identified as coming from Sunset Yellow). Thus, these different electrophoretic profiles illustrated the different qualitative composition of the lipsticks.

Comparison of the number and migration times of peaks was insufficient in the case of lipsticks L10 and L17, especially since the order of the identified peaks (those relating to a specific substance and those for a given color) was the same (see Fig. 4A). Electropherograms of both lipsticks were characterized by five distinct peaks at very similar migration times; they differed significantly but only in terms of the peak intensities. Thus, a semi-quantitative method of comparison was helpful here: not only the migration times, but also the ratios of the peak intensity were considered. Fig. 4B presents completely different graphs of individual peak heights at a given migration time for lipsticks L10 and L17. Therefore, it is possible to distinguish these two lipsticks due to the different amounts of components that migrated in similar times (the same compounds or of comparable chemical structure). Summarizing, every analysed lipstick was characterized by its own electrophoretic profile corresponding to the qualitative and quantitative composition characteristic for a given brand or series.

4. Conclusion

Microemulsion electrokinetic capillary chromatography is a technique with very high analytical potential. Based on the conducted research, it seems that it can be a useful tool in the examination of lipstick smears or imprints for forensic purposes. Due to the multitude of parameters that can be modified (not only the instrumental parameters of the separation process, but also the composition of the ME constituting the background electrolyte and thus the interactions occurring in the system during the separation process), MEEKC offers a wide range of possibilities for analyses of samples with such a complex composition as lipsticks.

It was found that the ME enabling best separation of the lipstick components contained 3% SDS, 6% butanol and 0.8% n-octane (w/w) in a borate buffer at pH 10. Short-end mode using a 30 cm capillary enabled the separation to be reduced to less than 4 min. UAE for 10 min

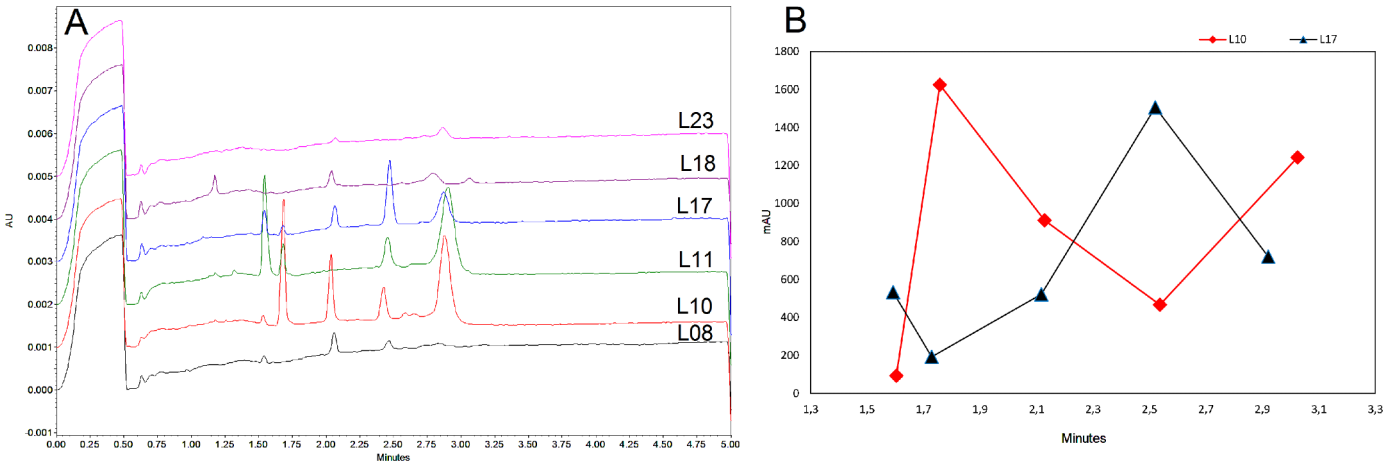

Fig. 4. A) Electropherograms at 220 nm obtained for the examined red lipsticks using ME2 in short-end mode: L08, L10, L11, L17, L18 and L23; B) Graphs of peak heights at a given migration time for L10 and L17 red lipsticks.

at 25 °C was found to be optimal for real lipstick samples preparation.

The UAE/MEEKC method optimized in this work was successfully applied to the analysis of several lipsticks with satisfactory repeatable results (intraday RSD_{tm} < 2.2%). A qualitative or semi-quantitative approach was sufficient for discrimination of the examined lipsticks. Generally speaking, in forensic chemistry investigations, in the vast majority of cases, comparative examinations are performed, i.e. evidence is compared with reference material, and expert reports are based on indicating differences or similarities between these materials. The developed MEEKC method has great potential to be a useful analytical tool in forensic laboratories.

It is worth noting, however, that further research is recommended to improve the developed UAE/MEEKC method. First of all, it is necessary to refine the sampling method to make the extraction more repeatable secondly, a study of lipstick aging (not only of lipstick traces, but also unused lipstick in its container) should be performed in order to verify how time and other conditions influence lipstick composition (and homogeneity). What is more, in case of real samples matrix effect depending on the place where the lipstick sample smear is found should be considered, and method improvements in this sense should be done. It is also intended to analyse a larger number of red lipstick samples as well as to check the applicability of the optimized method to lipsticks of other colors.

CRediT authorship contribution statement

Małgorzata Król: Conceptualization, Methodology, Investigation, Resources, Validation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition, Supervision. **Marlena Nowak:** Investigation, Formal analysis, Validation. **Marta Gładysz:** Conceptualization, Writing - review & editing, Visualization. **Paweł Kościelniak:** Writing - review & editing, Project administration.

Declarations of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.microc.2020.104735.

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